Regulation 3.2

The Walter and Eliza Hall Institute of Medical Research

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PROVISIONAL SPECIFICATION

for the invention entitled:

"A nucleic acid anchoring system"

The invention is described in the following statement:

A NUCLEIC ACID ANCHORING SYSTEM

FIELD OF THE INVENTION

The present invention relates generally to an anchoring system for nucleic acid molecules. The anchoring system generally comprises a solid support and a chemical linking moiety useful for chemical bond formation with another chemical moiety on a nucleic acid molecule. The present invention further contemplates methods for anchoring a nucleic acid molecule to a solid support *via* a covalent linkage. The anchoring system of the present invention is useful *inter alia* in construction of nucleic acid arrays, to purify nucleic acid molecules and to anchor nucleic acid molecules so that they can be used as templates for *in vitro* transcription and/or translation experiments, or as catalysts in polymerization reactions. The present invention is particularly adaptable for use with microspheres and the preparation of microsphere suspension arrays and optical fibre arrays. The anchoring system permits the generation of an anchored oligonucleotide for use as a universal nucleic acid conjugation substrate for any nucleic acid molecule or population of nucleic acid molecules. The present invention further provides a kit useful for anchoring nucleic acid molecules or comprising nucleic acid molecules already anchored to a solid support.

BACKGROUND OF THE INVENTION

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

The increasing sophistication of recombinant DNA technology is greatly facilitating research and development in a range of biotechnology-related industries.

Many manipulations involving nucleic acid molecules require immobilization strategies. One immobilization strategy involves the use of binding partners such as avidin and streptavidin. Whilst the latter system has been successfully employed in many nucleic acid

anchoring systems, it does have some limitations and does not enable the full gamit of nucleic acid manipulations now available to be performed on single and mixtures of nucleic acid molecules. It is also subject to non-specific binding thus limiting the accuracy of any immobilization reactions.

In addition, there are difficulties in using linker systems like streptavidin and avidin in automated and high throughput systems.

The immobilization procedure can be complex and involve the use of expensive reagents. There is a need, therefore, to develop a universal conjugation system for nucleic acid molecules.

In working leading up to the present invention, the subject inventor has developed a universal conjugation system for anchoring nucleic acid molecules to a solid support. The system of the present invention has a myriad of uses in molecular biology including micro or macro nucleic acid arrays, capturing, purifying and/or sorting nucleic acid molecules and microsphere nucleic acid technology. The system may also be usefully employed in high throughput and/or automated systems. In particular, the present invention provides a re-usable anchoring system for nucleic acid molecules.

SUMMARY OF THE INVENTION

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1, <400>2, etc. A sequence listing is provided after the claims.

The present invention provides a conjugation system for target nucleic acid molecules. The conjugation system facilitates immobilization or anchoring of the target nucleic acid molecules to a solid phase. The solid phase may be any form of solid support including microspheres, microchips, beads, slides such as glass slides, microtitre wells and dipsticks amongst many others.

The solid support is generally selected on the basis of ease of manipulation, inexpensiveness, thermal stability and stability to aqueous and/or organic solvents.

Silica and methacrylate microspheres are particularly useful especially for use in suspension arrays or optical fibre arrays.

The solid support is generally modified to include a chemical moiety capable of engaging in the formation of a covalent bond with another chemical moiety present on a nucleic acid molecule (the tag oligonucleotide). Any number of chemical moieties may be employed on the solid support but in a preferred embodiment, the solid support comprises a thiolated surface capable of engaging in covalent bond formation with an amine, thiol or acryl group linked to the 5' end of a tag oligonucleotide *via* a spacer molecule comprising C_n carbon atoms where n is from about 1 to about 100.

The level of success in anchoring the tag oligonucleotide to the solid support is measured by annealing an oligonucleotide which is complementary to the tag oligonucleotide (referred to herein as the "α-tag") optionally labeled with a reporter molecule such as but not limited to 6-FAM. The annealing of the α-tag results, in a preferred embodiment, in a 3' single-stranded overhang (or "sticky end") comprising the tag oligonucleotide.

Any target nucleic acid molecule is then ligated to the tag oligonucleotide *via* a bridging oligonucleotide. The bridging oligonucleotide comprises a sequence of nucleotides complementary to a nucleotide sequence of the 3' overhang portion of the tag oligonucleotide and a sequence of oligonucleotides complementary to a 5' end portion of a target nucleic acid molecule.

Accordingly, a target nucleic acid conjugating system is provided comprising a solid support having a tag oligonucleotide covalently bound to the surface of the solid support, the tag oligonucleotide rendered partially double-stranded by annealing an α-tag oligonucleotide to the tag oligonucleotide to provide a 3' overhang single-stranded portion of the tag oligonucleotide to which is annealed a bridging oligonucleotide having a nucleotide sequence capable of hybridizing to the 5' end portion of a target nucleic acid molecule.

In one embodiment, therefore, the present invention provides a universal nucleic acid anchoring system comprising the structure:-

$$S(-T)_p$$

wherein:

S is a solid support having a chemical moiety capable of covalent bond formation with a second chemical moiety;

T is a partially double-stranded oligonucleotide comprising a single-stranded

tag oligonucleotide having said second chemical moiety linked *via* a spacer molecule to its 5' end, said spacer comprising carbon atoms having the structure mC_n wherein n is the number of carbon atoms from about 1 to about 100, m is the number of repeats of C_n and ranging from about 1 to about 10; said tag oligonucleotide further comprising a complementary oligonucleotide (α-tag) annealed to the tag oligonucleotide to provide a 3' overhang or sticky end, single-stranded nucleotide sequence, on the tag oligonucleotide; said T further comprising a bridging oligonucleotide having a nucleotide sequence complementary to the 3' overhang nucleotide sequence on the tag oligonucleotide and a further nucleotide sequence complementary to a nucleotide sequence on the 5' end of a target nucleic acid molecule;

wherein T may be represented p times on the solid support wherein p is from about 1 to about 10,000.

In the above structure, the line "—" represents a covalent bond between a solid supoprt surface chemical moiety and the chemical moiety on the tag oligonucleotide.

The universal anchoring system of the present invention permits the generation of arrays of nucleic acid molecules. When the solid support comprises microspheres, the present invention permits the generation of suspension arrays. The anchored nucleic acid molecules may be subject to, for example, mutation identified or other manipulations such as *in vitro* transcription and/or translation reactions.

The nucleic acid anchoring system, i.e. $S(-T)_p$, may be re-used and, hence, only a single anchoring reaction need take place.

The present invention further contemplates a method for anchoring a target nucleic acid to a substrate, said substrate comprising:-

(i) a solid support having a surface chemical moiety;

(ii) a tag oligonucleotide having a chemical moiety linked to its 5' end *via* a spacer comprising a molecule with mC_n carbon atoms wherein n is the number of carbon atoms from about 1 to about 100 and m is the number of repeats of C_n from about 1 to about 100 wherein the latter chemical moity is in covalent bond formation with the chemical moiety on the surface of the solid support;

(iii) a complementary (α) tag oligonucleotide sequence which has hybridized to said tag oligonucleotide sequence such that there is a single-stranded nucleotide sequence constituting a 3' overhang of the tag oligonucleotide;

(iv) a bridging oligonucleotide having a complementary nucleotide sequence to the nucleotide sequence of the 3' overhang portion of the tag oligonucleotide and which bridging oligonucleotide has hybridized to its complementary sequence on the tag oligonucleotide leaving a single-stranded portion of the bridging oligonucleotide which has a complementary nucleotide sequence to the 5' terminal portion of said target nucleic acid molecule;

wherein said method comprises contacting said target nucleic acid molecule to said substrate for a time and under conditions to permit hybridization of the 5' portion of the nucleic acid molecule to the single-stranded portion of the bridging oligonucleotide and permitting ligase-mediated covalent bond formation between said target nucleic acid molecule and the substrate.

The following terms are used in the specification:-

TERM	DESCRPTION
tag oligonucleotide or tag	oligonucleotide molecule anchored to a solid support face *via* a covalent bond between a chemical moiety on the surface of the solid support and a chemical moiety conjugated to the oligonucleotide *via* a spacer molecule
α-tag	oligonucleotide molecule comprising a nucleotide sequence complementary to the tag oligonucleotide sequence
solid support	form of solid phase; includes microspheres, microchips, beads and slides
bridging oligonucleotide	oligonucleotide which bridges the tag oligonucleotide and the target nucleic acid molecule; the bridging oligonucleotide has a nucleotide sequence complementary to a 3' nucleotide sequence on tag and an end portion of the target nucleic acid molecule
spacer	a molecule comprising carbon atoms and having the structure mC_n wherein n is the number of carbon atoms and m is the number of repeats of C_n
target nucleic acid molecule	DNA or RNA target having a single-stranded end portion complementary to part of the bridging oligonucleotide
anchoring/anchored	joining of two molecules *via* a covalent linkage
chemical moiety	a chemical group capable of forming a covalent bond with another chemical moiety

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation of a carboxylated microsphere made from silica or methacrylate.

Figure 2 is a representation of a carboxylated bead with conjugated tag. The conjugation is made *via* an amidated oliogonucleotide and mediated by carbodiimide.

Figure 3 is a diagrammatic representation of carboxylated microsphere with a tag oligonucleotide with a complementary sequence (α-tag) leaving a 3' single-stranded overhang of the tag oligonucleotide.

Figure 4 is a diagrammatic representation showing (A) addition of bridging oligonucleotide to the partially double-stranded tag:α-tag complex; and (B) addition of target nucleic acid molecule anchored to the tag oligonucleotide *via* the bridging oligonucleotide.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides a nucleic acid anchoring system which facilitates ligase-mediated conjugation of a target nucleic acid molecule to a solid support *via* a tag oligonucleotide which is conjugated to the solid support *via* a covalent bond between a chemical moiety resident on the solid support and another chemical moiety on the tag nucleic acid molecule.

A first aspect of the present invention, therefore, is a tag oligonucleotide anchored to a solid support.

Accordingly, one aspect of the present invention provides a solid phase comprising a surface first chemical moiety capable of participating in covalent bond formation with a second chemical moiety conjugated to a tag oligonucleotide wherein the tag oligonucleotide is a substrate for ligase-mediated covalent bonding to a target nucleic acid molecule.

In one embodiment, the chemical moiety on the surface of the solid phase is capable of covalent bond formation with an amine group, a thiol group or an acryl group.

Alternatively, in another embodiment, the solid phase surface moiety is selected from an amine group, a thiol group or an acryl group which is capable of covalent bond formation with a chemical moiety such as a carboxyl group on a tag oligonucleotide.

Accordingly, another aspect of the present invention is directed to a solid phase comprising a surface first chemical moiety selected from a carboxyl group, an amine group, a thiol group and an acryl group, said first chemical moiety capable of participating in covalent bond formation with a second chemical moiety selected from a carboxyl group, an amine group, a thiol group and an acryl group conjugated to an oligonucleotide with the proviso that when the solid phase surface moiety is a carboxyl group then the covalent bond forms with an amine group, a thiol group or an acryl group. The present invention extends,

however, to chemical moieties capable of any form of covalent bond formation with any other chemical entity.

In one preferred embodiment, the chemical moiety on the surface of the solid phase is a thiol group and such a group is capable of covalent bond formation with a number of chemical moieties such as one of an amine group, a thiol group or an acryl group and when the solid phase chemical moiety is one of an amine group, a thiol group or an acryl group then the covalent bond is formed with a carboxyl group on the tag oligonucleotide.

In a most preferred embodiment, the solid phase surface chemical moiety is a thiol group.

Accordingly, another aspect of the present invention is directed to a solid phase comprising a surface carboxyl group capable of participating in covalent bond formation with a chemical moiety selected from an amine group, a thiol group and an acryl group conjugated to a tag oligonucleotide.

Most preferably, the chemical moiety conjugated to the tag oligonucleotide is an acryl group.

In this embodiment of the present invention, there is provided a solid phase comprising a surface carboxyl group capable of participating in covalent bond formation with an amine group conjugated to a tag oligonucleotide.

The solid phase is preferably in the form of a solid support such as a microsphere, bead, glass, ceramic or plastic slide, a dipstick or the wall of a vessel such as a microtiter well. The form of the solid support is not critical and may vary depending on the application intended. However, microspheres such as silica or methacrylate microspheres are particularly useful in the practice of the present invention, especially for use in suspension arrays or optical fiber arrays.

The selection of solid supports is conveniently based on ease of manipulation, level of

expense, thermal stability and/or stability in aqueous and/or organic solvents.

In a particularly preferred embodiment, therefore, the present invention is directed to microspheres having a carboxylated surface capable of participating in covalent bond formation with a chemical moiety selected from an amine group, a thiol group and an acryl group conjugated to a tag oligonucleotide.

Generally, any number of chemical moieties may be present or exposed on the surface of the solid support and these may range from a few hundred to several thousand.

In a particularly preferred embodiment, there are from about 3,000 to about 10,000 surface chemical moieties potentially involved in covalent bonding per solid support. This is particularly the case when the sold support is a microsphere. Conveniently, the microsphere comprises from about 4,000 to about 8,000 or more conveniently from about 5,000 to about 7,000 chemical moieties per bead.

In relation to one preferred embodiment, therefore, the present invention provides microspheres each comprising from about 3,000 to about 10,000 such as about 4,000 to about 8,000 or more particularly about 3,000 to about 5,000 surface carboxyl groups per microsphere.

The tag oligonucleotide having the chemical moiety capable of covalent bond formation with the solid phase surface chemical moiety may comprise any nucleotide sequence although the nucleotide sequence would generally be known. One particularly useful sequence is an RNA polymerase promoter nucleotide sequence such as the SP6 RNA polymerase promoter nucleotide sequence. The benefit of the latter in terms of linking DNA is the ability to generate RNA transcripts. However, any oligonucleotide of known sequence may be employed. The term "oligonucleotide" is not to be viewed to any limiting extent and may comprise from about 10 base pairs (bp) to hundreds of bp.

It is convenient to ensure that after binding of the tag oligonucleotide to the solid phase

that the tag oligonucleotide does not exhibit interference with the solid support surface. Consequently, a spacer molecule is generally included between the chemical moiety and the 5' end of the tag oligonucleotide. A spacer comprising carbon-based molecules such as having from about C_1 to about C_{100} carbon atoms, more preferably from about C_{10} to about C_{50} and even more preferably from about C_{18} to about C_{36} is particularly useful.

The spacer may also be multiple repeats such as 2 x C_{18} spacers or 3 x C_6 spacers. The length of the spacer is not critical and may be varied depending on the intended application.

Consequently, another aspect of the present invention contemplates an isolated tag oligonucleotide comprising a chemical moiety capable of covalent bond formation with a chemical moiety on the surface of a solid phase, said first mentioned chemical moiety conjugated to said tag oligonucleotide *via* a carbon molecule having mC_n carbon atoms wherein C is a carbon atom, n is the number of carbon atoms and m is the number of repeats of C_n molecules and is 1 or greater than 1.

Generally, n is from about 1 to about 100 and m is preferably 1 or from about 2 to about 10.

Conveniently, the total number of carbon atoms is from about 20 to about 50.

The spacer molecule is conveniently an alkyl, alkenyl or an alkynyl molecule. Preferably, the spacer is a linear non-branched hydrocarbon although any other molecule may be employed to separate the oligonucleotide from the surface of the solid support.

The 5' tag oligonucleotide chemical moiety is conveniently an amine group, a thiol group or an acryl group if the solid support surface chemical moiety is an carboxyl group. Alternatively, the 5' chemical moiety is a carboxyl group and the solid phase surface chemical moiety is one or more of an amine group, a thiol group and/or an acryl group.

In a most preferred embodiment, the 5' chemical moiety on the tag oligonucleotide is an amine group.

In accordance with the above aspect of the present invention, the solid support is preferably a microsphere although any solid support may be employed.

Accordingly, another aspect of the present invention provides a solid phase comprising a tag oligonucleotide anchored to the surface of said solid phase *via* a covalent bond between a chemical moiety on the surface of the solid phase and a chemical moiety conjugated to said tag oligonucleotide *via* a carbon atom (C) spacer having the structure mC_n wherein n is the number of carbon atoms from about 1 to about 100 and m is the number of repeats of the Cn molecules and is from about 1 to about 10.

As indicated above, the covalent bond is conveniently a carboxyl group covalently bonded to an amine, thiol or acryl group. Furthermore, the carbon atom containing molecule is preferably from about 20 to about 50 carbon atoms in length.

Consequently, another aspect of the present invention comprises an article of manufacture having the structure:-

$$S-mC_n-[x_1x_2...x_p]$$

wherein:

S is a solid support;

C is a carbon atom

n is the number of carbon atoms and is from about 1 to about 100;

m is the number of repeats of the C_n moieties and is from about 1 to about 10;

and

$[x_1x_2 \ldots x_p]$ is a nucleotide sequence of nucleotides $x_1x_2 \ldots x_p$ wherein each of $x_1x_2 \ldots x_p$ may be the same or different and the nucleotide length, p, is from 5 to about

200.

In the above formation, the schematic "—" represents a covalent bond such as, for example, an amide bond.

The oligonucleotide sequence, i.e. $x_1x_2 \ldots x_p$ is any known sequence such as the SP6 RNA polymerase promoter. The oligonucleotide sequence may also comprise an additional nucleotide sequence having, for example, translation start signals, ribosome binding sites and an initial common triplet.

It is particularly convenient to ensure or to measure successful covalent attachment of the tag oligonucleotide sequence to the solid phase. This can be accomplished by incorporating a complementary internally labeled oligonucleotide sequence. Conveniently, the internally labeled oligonucleotide sequence is complementary to the 5' end of the anchored tag oligonucleotide sequence. The internal label may be any suitable label such as 6-FAM at its 3' end. The 5' end is generally phosphorylated.

Accordingly, another aspect of the present invention provides a solid phase comprising a tag oligonucleotide of known sequence anchored thereto *via* a covalent linkage between a chemical moiety on the surface of the solid phase and a chemical moiety conjugated to the tag oligonucleotide *via* a molecule of n carbon atoms wherein n is from about 1 to about 100, said solid phase further comprising a second oligonucleotide sequence annealed by base pairing to a complementary nucleotide sequence on said first mentioned tag oligonucleotides resulting in an overhang at the 3' end of either the tag oligonucleotide or its complementary oligonucleotide.

Preferably, the second oligonucleotide sequence comprises a label and is used to measure the success or otherwise of the covalent anchoring of the first oligonucleotide sequence to the solid phase.

The preferred label is 6-FAM.

Preferably, the first oligonucleotide sequence overhangs at its 3' end over the second oligonucleotide sequence.

As indicated above, the second oligonucleotide is labeled and, hence, it becomes a convenient assay for the success or otherwise of covalent attachment of the first oligonucleotide to the solid phase. One skilled in the art will immediately rcognize that there are many variations in order to determine the extent of covalent linkage and that the present invention should not be only limited to one particular means.

The essence of this aspect of the invention is a solid phase having a first tag oligonucleotide attached thereto *via* covalent linkage between a first chemical moiety on the surface of the solid phase (e.g. a carboxyl group) and a second chemical moiety conjugated to the first oligonucleotide *via* a spacer molecule of length mC_n as defined above and a second tag oligonucleotide, optionally labeled with a reporter molecule capable of giving an identifiable signal, which anneals to complementary nucleotide sequences on the first oligonucleotide to provide, in a preferred embodiment, a 3' overhang of the first tag oligonucleotide and wherein the 5' end of the second tag oligonucleotide is phosphorylated.

The complementary oligonucleotide to the tag oligonucleotide is referred to herein as α-tag or the α-tag oligonucleotide.

The present invention provides, therefore, in one embodiment:-

(i) a solid phase such as a microsphere, microchip or the sides of a well in a microtiter plate; and

(ii) a tag oligonucleotide having a chemical moiety conjugated to the oligonucleotide *via* a molecule of mC_n carbon atoms as described above;

wherein the chemical moiety on the oligonucleotide is in covalent bond formation with a chemical moiety on the surface of the solid phase.

Again, as stated above, although a covalent linkage such as an amide bond is particularly useful in the practice of the present invention, it is but one of a whole myriad of covalent linkages which may be used in accordance with the present invention.

The advantage of the covalent linkage between tag and solid phase is that it need only be performed once. The solid support comprising the tag oligonucleotide may then be used any number of times.

The above solid support generally further comprises a second oligonucleotide (α-tag) in complementary base pairing to the first mentioned oligonucleotide (tag) such that there is optionally a label on the 3' end of the α-tag oligonucleotide and the 5' end is phosphorylated wherein the tag oligonucleotide overhangs the α-tag oligonucleotide at the 3' end of the tag oligonucleotide.

The next step is the generation of a bridge oligonucleotide which enables anchoring of a target nucleic acid molecule to the tag oligonucleotide anchored to the solid phase.

The bridging oligonucleotide, in the case where the tag oligonucleotide overhangs at its 3' end relative to the annealed α-tag oligonucleotide, anneals in a 3' → 5' direction where the 3' end is complementary to the overhanging portion of the tag oligonucleotide.

The 5' end of the bridge is then complementary to an end portion of a target nucleic acid molecule. Both the 5' end of the target nucleic acid molecule and the 5' end of the labeled α-tag oligonucleotide (complementary to the anchored tag oligonucleotide) are phosphorylated. A ligase-mediated covalent attachment then forms anchoring the target nucleic acid molecule to the anchored tag *via* the bridging oligonucleotide.

Accordingly, in one embodiment, there is provided a substrate for anchoring a target

nucleic acid molecule, said substrate comprising:-

(i) a solid phase having a first chemical moiety on its surface;

(ii) a tag oligonucleotide comprising a second chemical moiety in covalent bond formation with the first chemical moiety, said second chemical moiety conjugated to the tag oligonucleotide *via* a molecule of structure mC_n as defined above;

(iii) an optionally labeled oligonucleotide complementary to the tag oligonucleotide resulting in a 3' singled-stranded overhang of the tag oligonucleotide; and

(iv) a bridging oligonucleotide having complementary based to the 3' overhang region of the tag oligonucleotide and complementary bases to the 5' end portion of the target nucleic acid molecule wherein the target nucleic acid molecule is anchored to the tag oligonucleotide *via* ligase-mediated conjugation.

The bridging oligonucleotide may be part of the solid phase complex prior to anchoring of the target nucleic acid molecule or it may be first added to and annealed to the target nucleic acid molecule prior to annealing to the tag oligonucleotide.

Yet in a further embodiment, the solid phase-tag oligonucleotide complex, the bridging oligonucleotide and the target nucleic acid molecule are admixed together and subjected to ligation conditions.

The target nucleic acid molecule is specific for each conjugation experiment. Generally, its initial 5-30 bases are complementary to the bases at the 3' end of the tag oligonucleotide. The 5' end of the target nucleic acid molecule is generally phosphorylated. A minimum of 5 bases complementary between the target nucleic acid molecule and the tag oligonucleotide is enough to enable ligation but generally insufficient to permit cross-hybridization, especially when multiplexing a large number of target molecules.

Yet another aspect of the present invention provides a universal nucleic acid anchoring system comprising the structure:-

$$S(-T)_p$$

wherein:

S is a solid support having a chemical moiety capable of covalent bond formation with a second chemical moiety;

T is a partially double-stranded oligonucleotide comprising a single-stranded tag oligonucleotide having said second chemical moiety linked *via* a spacer molecule to its 5' end, said spacer comprising carbon atoms having the structure mC_n wherein n is the number of carbon atoms from about 1 to about 100, m is the number of repeats of C_n and ranging from about 1 to about 10; said tag oligonucleotide further comprising a complementary oligonucleotide (α-tag) annealed to the tag oligonucleotide to provide a 3' overhang or sticky end, single-stranded nucleotide sequence, on the tag oligonucleotide; said T further comprising a bridging oligonucleotide having a nucleotide sequence complementary to the 3' overhang nucleotide sequence on the tag oligonucleotide and a further nucleotide sequence complementary to a nucleotide sequence on the 5' end of a target nucleic acid molecule;

wherein T may be represented p times on the solid support wherein p is from about 1 to about 10,000.

Still another aspect of the present invention contemplates a method for immobilizing a target nucleic acid molecule to a partially double-stranded tag oligonucleotide anchored to a solid support, said method comprising ligating a phosphorylated 5' end of the target nucleic acid molecule to a complementary single-stranded portion of the tag oligonucleotide under conditions to permit ligase-mediated covalent bond formation wherein said tag oligonucleotide is covalently anchored to the solid support *via* covalent

bond formation between a first chemical moiety on the surface of the solid support and a chemical moiety conjugated to the tag oligonucleotide *via* a molecule of structure mC_n as defined above and wherein the tag oligonucleotide is rendered partially double-stranded by annealing a complementary oligonucleotide to the tag oligonucleotide leaving a single-stranded 3' terminal portion of the tag oligonucleotide which is used to capture the target nucleic acid molecule *via* a bridging oligonucleotide.

The present invention further provides a kit useful in capturing and/or anchoring target nucleic acid molecules. The kit is conveniently in multi-compartment form wherein a first compartment comprises a solid support such as microspheres or microchips having a surface chemical moiety. A second compartment comprises a tag oligonucleotide having a chemical moiety capable of covalent bond formation with the surface chemical moiety of the solid support and wherein the chemical moiety on the tag is linked to the tag *via* a molecule of the mC_n structure as defined above. A third compartment comprises a labeled complementary tag oligonucleotide and a fourth compartment comprises a bridging oligonucleotide.

In an alternative, the kit may comprise a solid support having a partially double-stranded tag oligonucleotide anchored thereto comprising a single-stranded 3' end portion. The kit may then have a bridging oligonucleotide already attached to the single-stranded portion of the tag oligonucleotide or this may be maintained separately. A target nucleic acid molecule is then ligated to the tag oligonucleotide *via* the bridge oligonucleotide.

The anchoring system of the present invention has many uses such as in deconvolution of complex mixtures of nucleic acid molecules, sorting of nucleic acid molecules and for generation of microarrays, suspension arrays and optical fiber arrays.

The system may also be adopted to facilitating *in vitro* transcription and/or translation and the transcription and/or translation products assayed or used to screen for ligand or binding partners.

The anchoring system of the present invention may be fully or partially automated and may be used for high throughput screening of target nucleic acid molecules.

The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

Selection of components of anchoring systems

1. Solid support

The physico-chemico structure of the surface of the solid support is an important consideration for the choice of chemical reactive moiety of the DNA to exploit for covalent attachment. The main attributes of the surface are:-

(a) ease of manipulation;
(b) inexpensive;
(c) stable in extremes of temperatures; and
(d) stable in both aqueous and organic solvents.

Suitable surfaces include glass slides for solid microarrays and silica and methacrylate microspheres for use in suspension arrays or optical fiber arrays. The one favoured at the moment and representing the most common conjugation chemistry involves a carboxylated surface is exemplified below.

2. A universal tag for initial modification of the surface

In the present system, a reactive end (amine, thiol or acryl group) is used at the 5' end of the DNA oligonucleotide. In the example given here, the 5' reactive group is an amine, followed by two C18 spacers. These additions are made at the point of synthesis.

To this common 5' end, architecture is added to a 20 base linker designed on the T7 RNA polymerase promoter along with an additional 3 bases. A longer and more adaptable common tag may also be used involving translation start signals, ribosome binding sites and initial common triplets.

The universal tag comprises the structure:-

5'-NH2-C18-C18-TAATACGACTCACTATAGGGCGA [SEQ ID NO:1]

3. A labeled α-tag

To assay the successful covalent attachment of the tag to the surface, a complementary internally labeled 16-mer built to bind to the first 16 bases of the tag is used. The 3' end is fluoresceinated with 6-FAM and the 5' end is posphorylated.

The sequence of the complementary tag is as follows:-

5'PO4-ATAGTGAGTCGTATTA-FAM [SEQ ID NO:2]

4. A bridge oligo

This bridge is built to be complementary to the last 6 bases of the tag as well as the first 5 bases of the target. It is kept small for easy removal from reactions, but long enough to be easily scored by electrophoresis. The bridge needs no 5' modifications.

Its structure is:-

5'-TCCCGCTCCTAGA [SEQ ID NO:3]

5. Phosphorylated target

This DNA is made to be specific for each experiment. It has its initial 5 bases reverse complementary to the 57' 5 bases of the bridge. The 5' end is phosphorylated. The 5 bases which are common the 5' end are sufficient to enable ligation, but not sufficient enough to significantly add to cross-hybridization and high background when multiplexing large numbers of target. In the present system test, the 3' end of the target contained the reverse complement of the SP6 RNA polymerase promoter allowing for either translation or, in

concert with T7 promoter, PCR amplication.

An example of a target is as follows:

5'-PO4-GGATCTGACACGGACTGATGAATTCC-Asp6-3' [SEQ ID NO:4]

EXAMPLE 2

System Set-up

1. ***Tag is conjugated to surface***

The execution of this step depends on the chemistry and surface used. The assay for measurement of amount of covalent binding is performed by binding α-tag to the solid surface. Amount of fluorescence at 521 nM is measured after excitation at 511 nM by a high energy light source. The argon ion laser of the ABI 377, ABI 3700 or BD facscalibur may conveniently be used to measure this quantity.

2. ***Target is ligated to tag by bridging ligation***

The bridge and target are added in equimolar amounts to the tat:α-tag modified surface with T4 DNA ligase. Successful ligation of target to tag is measured indirectly by measuring the ligation of α-tag to bridge electrophoretically (a 27-mer *vs* an 11-mer and a 16-mer).

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

CLAIMS

1. A solid phase comprising a surface first chemical moiety capable of participating in covalent bond formation with a second chemical moiety conjugated to a tag oligonucleotide wherein the tag oligonucleotide is a substrate for ligase-mediated covalent bonding to a target nucleic acid molecule.

2. The solid phase of Claim 1 comprising a solid support in the form of a microsphere, microchip or a glass, plastic or ceramic slide.

3. The solid phase of Claim 2 wherein the solid support is a microsphere.

4. The solid phase of Claim 1 wherein the surface chemical moiety is capable of covalent bond formation with an amine group, a thiol group or an acryl group.

5. The solid phase of Claim 1 wherein the surface chemical moiety is capable of covalent bond formation with a carboxyl group.

6. The solid phase of Claim 1 wherein the surface chemical moiety is a carboxyl group.

7. The solid phase of Claim 1 wherein the second chemical moiety is an amine group.

8. The solid phase of Claim 1 wherein the tag oligonucleotide comprises a chemical moiety conjugated to a known oligonucleotide sequence *via* a molecule comprising carbon atoms having the formula mC_n wherein n is the number of carbon atoms from about 1 to about 100 and m is the number of times C_n is repeated and is from about 1 to about 10.

9. The solid phase of Claim 8 further comprising an oligonucleotide (α-tag)

which is complementary binding to said tag oligonucleotide such that there is a 3' overhang of said tag oligonucleotide.

10. The solid phase of Claim 9 wherein the α-tag oligonucleotide is labeled with a reporter molecule and is phosphorylated at its 5' end.

11. The solid phase of Claim 9 or 10 or further comprising a bridging oligonucleotide, said bridging oligonucleotide having a nucleotide sequence complementary to the nucleotide sequence of the 3' overhang portion of the tag oligonucleotide and a nucleotide sequence complementary to a terminal end portion of a target nucleic acid molecule.

12. The solid phase of Claim 11 further comprising a target nucleic acid molecule in ligase-mediating covalent bonding to the α-tag oligonucleotide molecule anchored to the solid phase.

13. A substrate for anchoring a target nucleic acid molecule, said substrate comprising:-

(i) a solid phase having a first chemical moiety on its surface;

(ii) a tag oligonucleotide comprising a second chemical moiety in covalent bond formation with the first chemical moiety, said second chemical moiety conjugated to the tag oligonucleotide *via* a molecule of structure mC_n as defined above;

(iii) an optionally labeled oligonucleotide complementary to the tag oligonucleotide resulting in a 3' singled-stranded overhang of the tag oligonucleotide; and

(iv) a bridge oligonucleotide having complementary based to the 3' overhang region of the tag oligonucleotide and complementary bases to the 5' end

stranded portion of the tag oligonucleotide under conditions to permit ligase-mediated covalent bond formation wherein said tag oligonucleotide is covalently anchored to the solid support *via* covalent bond formation between a first chemical moiety on the surface of the solid support and a chemical moiety conjugated to the tag oligonucleotide *via* a molecule of structure mC_n as defined above and wherein the tag oligonucleotide is rendered partially double-stranded by annealing a complementary oligonucleotide to the tag oligonucleotide leaving a single-stranded 3' terminal portion of the tag oligonucleotide which is used to capture the target nucleic acid molecule *via* a bridging oligonucleotide.

SEQUENCE LISTING

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<120> A nucleic acid anchoring system

<130> 2521653/EJH

<140> Not yet available
<141> 2002-06-04

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DATED this fourth day of June 2002.

The Walter and Eliza Hall Institute of Medical Research

by DAVIES COLLISION CAVE

Patent Attorneys for the Applicant

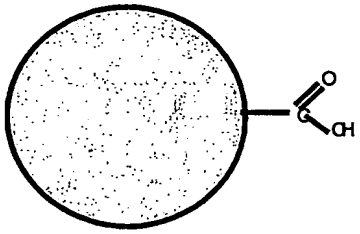

Figure 1

Figure 2

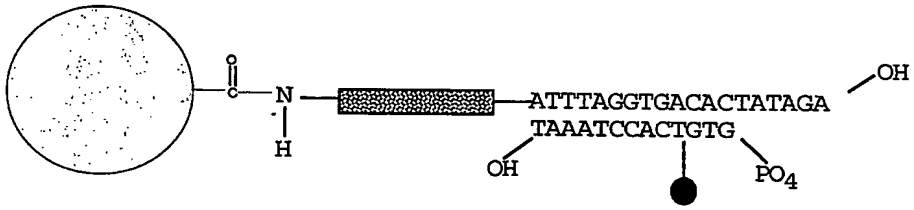

Figure 3

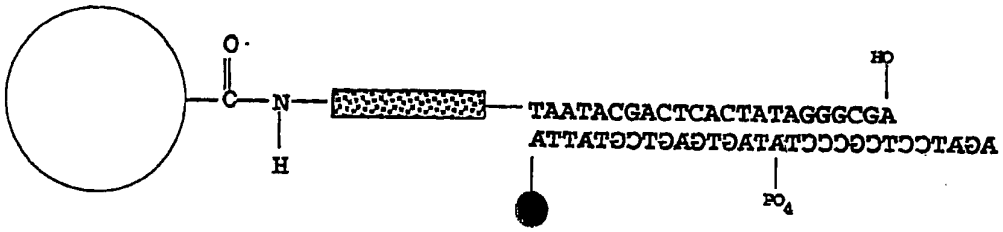

Figure 4A

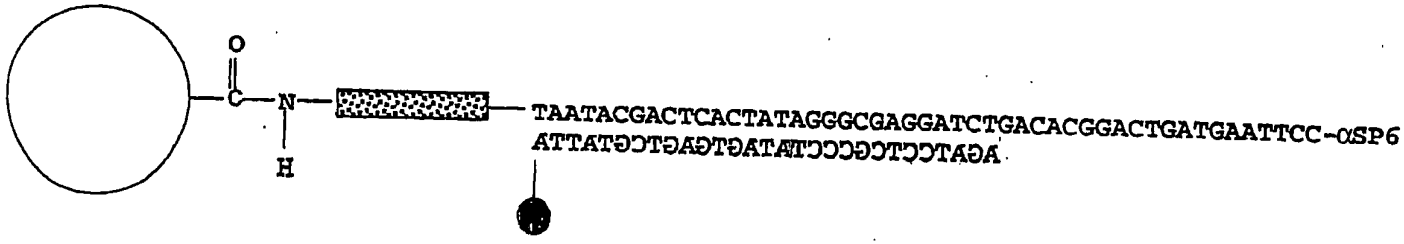

Figure 4B